Monolith Protocol MO-P-068

TGT Dimerization

Shigella bacteria constitute the causative agent of bacillary dysentery, an acute inflammatory disease causing the death of more than one million humans per year. The enzyme tRNA-guanine transglycosylase (TGT) catalyzes the base exchange of the genetically encoded guanine at the wobble position of particular tRNAs by the modified base preQ1. Further modification of preQ1 and the final incorporation of queuine into the tRNAs lead to the development of the virulence phenotype of *Shigella*, thus making TGT a suitable drug target. The enzyme is biologically active only as a homodimer, as one monomer subunit performs the catalytic base-exchange reaction while the second subunit is required to position the tRNA substrate in the correct orientation.

protein dimerization | homodimer

A1. Target/Fluorescent Molecule

Queuine tRNA-ribosyltransferase (TGT)
uniprot.org/uniprot/P28720

A2. Molecule Class/Organism

tRNA-ribosyltransferase
Zymomonas mobilis subsp. mobilis (strain ATCC 31821 / ZM4 / CP4)

A3. Sequence/Formula

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GSMVEATAQE TDRPRFSFSI AAREGKARTG TIEMKRGVIR TPAFMPVGTA ATVKALKPET VRATGADIIL GNTYHLMLRP
GAERIAKLGG LHSFMGWDRP ILTDSGGYQV MSLSSLTKQS EEGVTFKSHL DGSRHMLSPE RSIEIQHLLG SDIVMAFDEC
TPYPATPSRA ASSMERSMRW AKRSRDAFDS RKEQAENAAL FGIQQGSVFE NLRQQSADAL AEIGFDGYAV GGLAVGEGQD
EMFRVLDFSV PMLPDDKPHY LMGVGKPDDI VGAVERGIDM FDCVLPTRSG RNGQAFTWDG PINIRNARFS EDLKPLDSEC
HCAVCQKWSR AYIHXLIRAG EILGAMLMTE HNIAFYQQLM QKIRDSISEG RFSQFAQDFR ARYFARNS
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wt X = H

H333D X = D

A4. Purification Strategy/Source

University of Marburg (Prof. Gerhard Klebe)[1]

A5. Stock Concentration/Stock Buffer

wt	*H333D*
8.54 mg/ml | 199 µM	7.76 mg/ml | 180 µM
10 mM HEPES, pH 7.4, 1 M NaCl	10 mM HEPES, pH 7.4, 1 M NaCl

[1] Jakobi et al., ACS Chem. Biol., 10, 8, 1897-1907 (2015)

A6. Molecular Weight/Extinction Coefficient

wt	*H333D*
43,013.8 Da	42,991.8 Da
33,920 $M^{-1}cm^{-1}$ (ε_{280})	33,920 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

10 mM HEPES, pH 7.4, 1 M NaCl, 0.005% TWEEN® 20

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS 2nd Generation (MO-L011, NanoTemper Technologies GmbH)
1* Dye RED-NHS 2nd Generation (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Prepare 100 µL of a 10 µL TGT solution in dilution buffer.
2. Add 25 µL of DMSO to 10 µg RED-NHS 2nd Generation dye to obtain a ~600 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 5 µL of the 600 µM dye solution with 95 µL of dilution buffer to obtain 100 µL of a 30 µM dye solution (3x protein concentration).
4. Mix TGT and dye in a 1:1 volume ratio (200 µL final volume, 2.5% final DMSO concentration).
5. Incubate for 30 minutes at room temperature in the dark.
6. In the meantime, remove the top cap of the B-Column and pour off the storage solution. Remove the bottom cap and place with adapter in a 15 mL tube.
7. Fill the column with dilution buffer and allow it to enter the packed resin bed completely by gravity flow. Discard the flow through collected. Repeat this step 3 more times.
8. Add 200 µL of the labeling reaction from step 4 to the center of the column and let sample enter the resin bed completely.
9. Add 500 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 500 µL of dilution buffer and collect the eluate.
11. Keep the labeled TGT (~2 µM) on ice in the dark.

A10. Labeling Efficiency

Measurement of protein concentration and degree of labeling (DOL) using a NanoDrop™:
nanotempertech.com/dol-calculator

wt

Absorbance A_{205}	1.66	Protein concentration	1.2 µM
Absorbance A_{650}	0.05	Degree-of-labeling (DOL)	0.29

H333D

Absorbance A_{205}	1.54	Protein concentration	1.1 µM
Absorbance A_{650}	0.07	Degree-of-labeling (DOL)	0.49

B1. Ligand/Non-Fluorescent Binding Partner

Queuine tRNA-ribosyltransferase (TGT)
uniprot.org/uniprot/P28720

B2. Molecule Class/Organism

tRNA-ribosyltransferase
Zymomonas mobilis subsp. mobilis (strain ATCC 31821 / ZM4 / CP4)

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B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Add 20 µL of 40 µM TGT into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
2. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
3. Mix 2 µL of labeled TGT (~2 µM) with 198 µL of dilution buffer to obtain 200 µL of ~20 nM TGT.
4. Add 10 µL of labeled TGT (~20 nM) to each tube from **16** to **1** and mix by pipetting.
5. Incubate tubes for at least 30 minutes[3] at room temperature in the dark before loading capillaries.

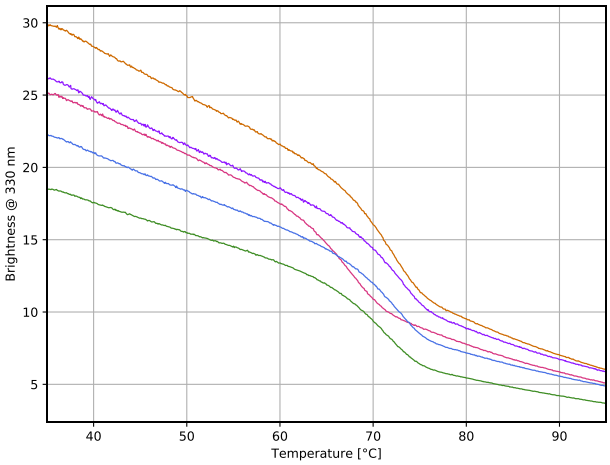
[2] Jakobi et al., ACS Chem. Biol., 10, 8, 1897-1907 (2015)

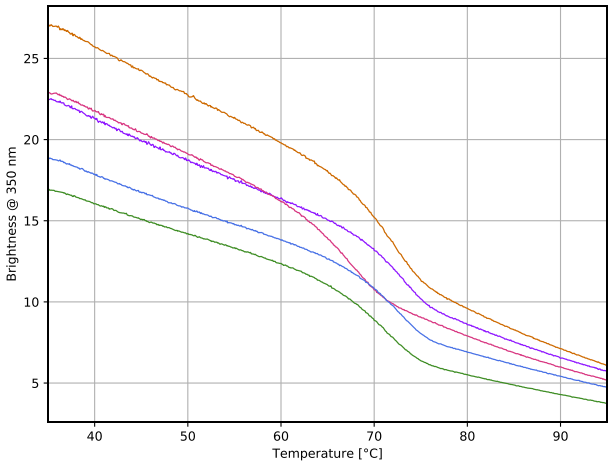
[3] As the decay of stable dimers can be very slow, longer incubation times may be required to reach complete equilibrium.

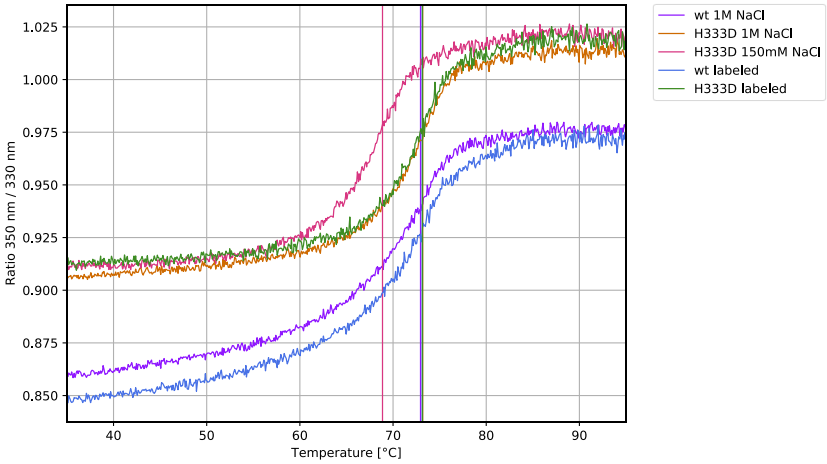
C. Applied Quality Checks

Validation of structural integrity of unlabeled and labeled TGT in low and high salt buffers using Tycho NT.6: nanotempertech.com/tycho

wt 1M NaCl	10 µL of 2 µM *wt* in dilution buffer	T_i = 72.9°C
H333D 1M NaCl	10 µL of 2 µM *H333D* in dilution buffer	T_i = 73.2°C
H333D 150 mM NaCl	10 µL of 2 µM *H333D* in 10 mM HEPES, pH 7.4, 150 mM NaCl	T_i = 68.9°C
wt labeled	10 µL of *wt* B-column eluate (~2 µM)	T_i = 73.1°C
H333D labeled	10 µL of *H333D* B-column eluate (~2 µM)	T_i = 73.2°C







D1. MST System/Capillaries

Monolith NT.115^{Pico} Red (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

D2. MST Software

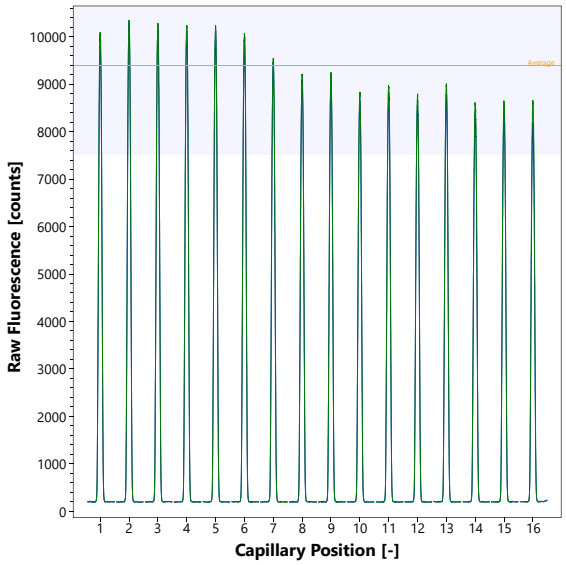
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

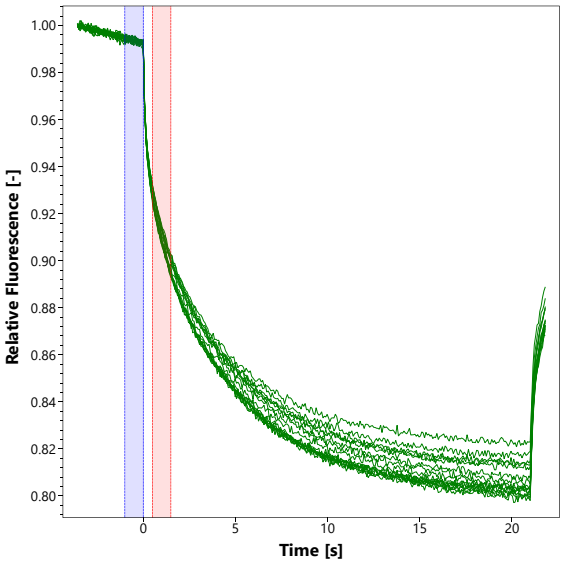
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

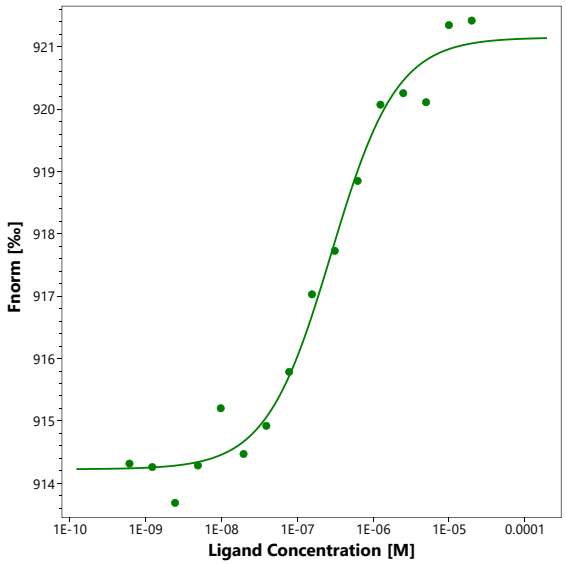
10 mM HEPES, pH 7.4, 1 M NaCl, 0.005% TWEEN® 20
10 nM (labeled) TGT | 20 µM – 0.6 nM (unlabeled) TGT | 24°C | medium MST power | 20% excitation power

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

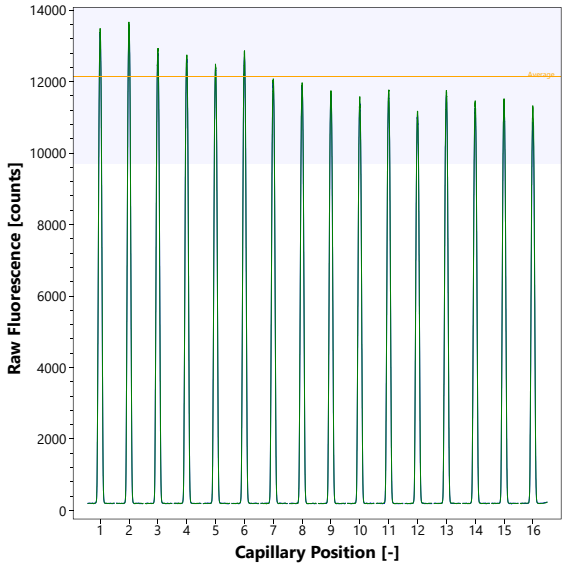
K_d = 202 ± 33 nM (*wt*)

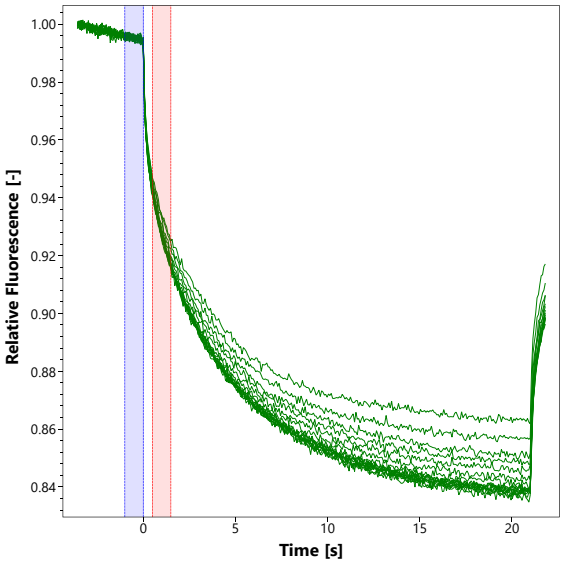


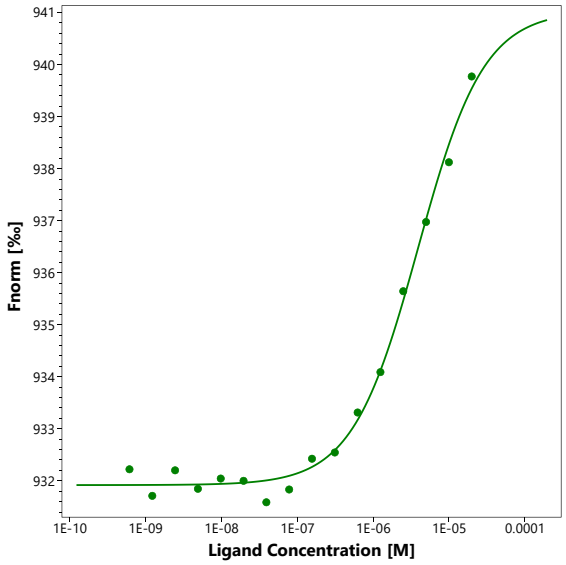




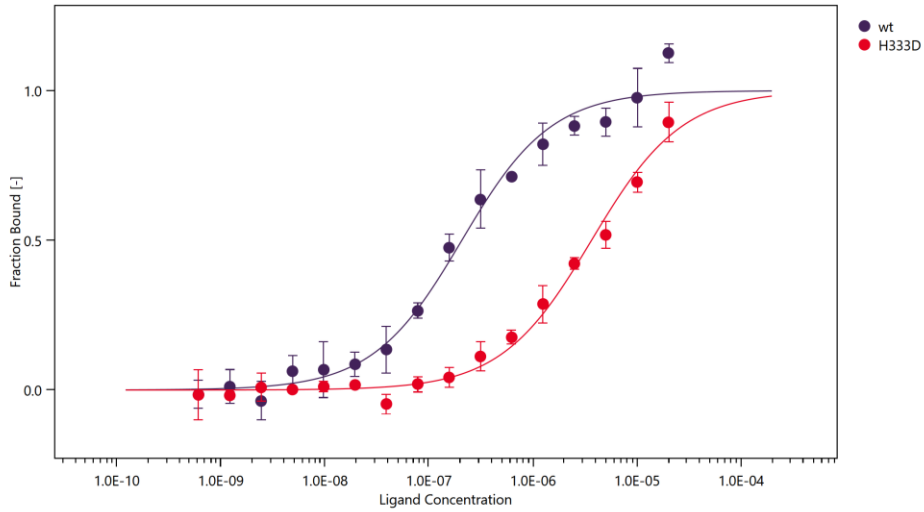
K_d = 3.6 ± 0.6 µM (*H333D*)







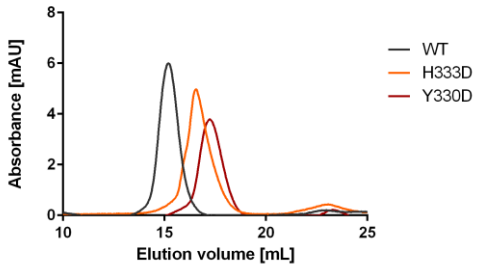
Comparison of dimerization affinities *wt* vs. *H333D* (n = 3):



D5. Reference Results/Supporting Results

10 µM *wt*:	2% monomer / 98% dimer	
10 µM *H333D*:	58% monomer / 42% dimer	Native MS
10 µM *Y330D*:	99% monomer / 1% dimer	Jakobi et al., ACS Chem. Biol. 2015, 10, 8, 1897-1907 (2015)

Analytical SEC:



E. Contributors

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